Supplementary Information

Automated model-predictive design of synthetic promoters to control transcriptional profiles in bacteria

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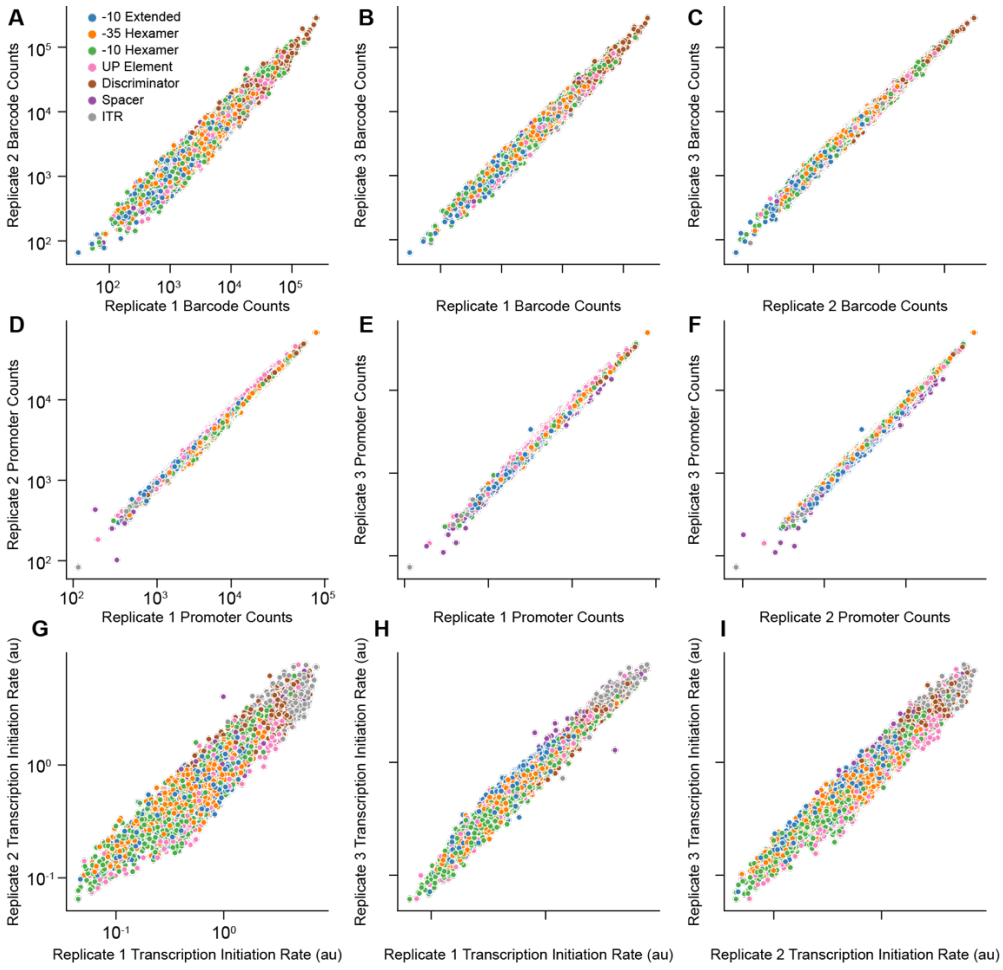
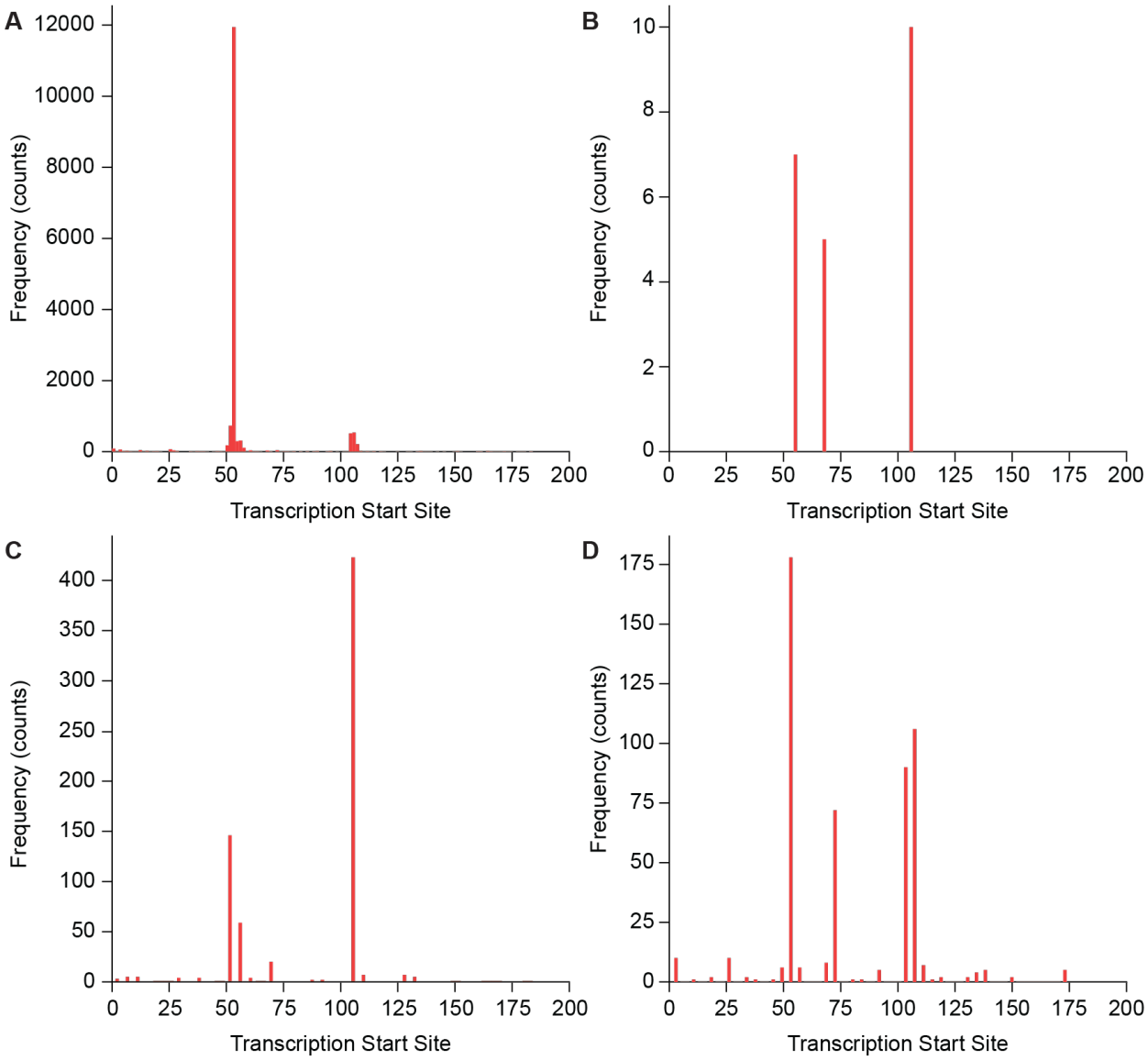
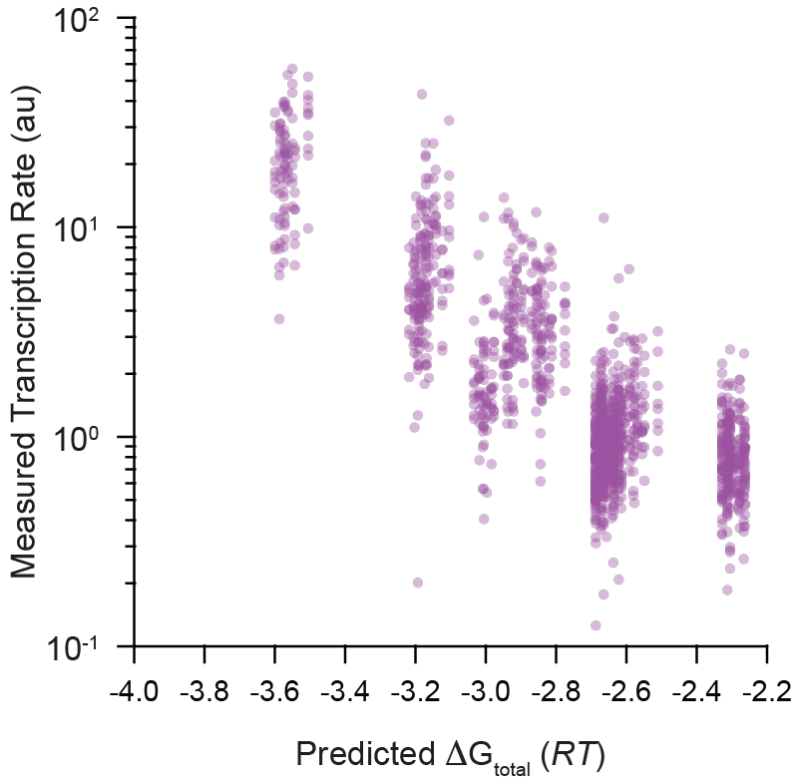


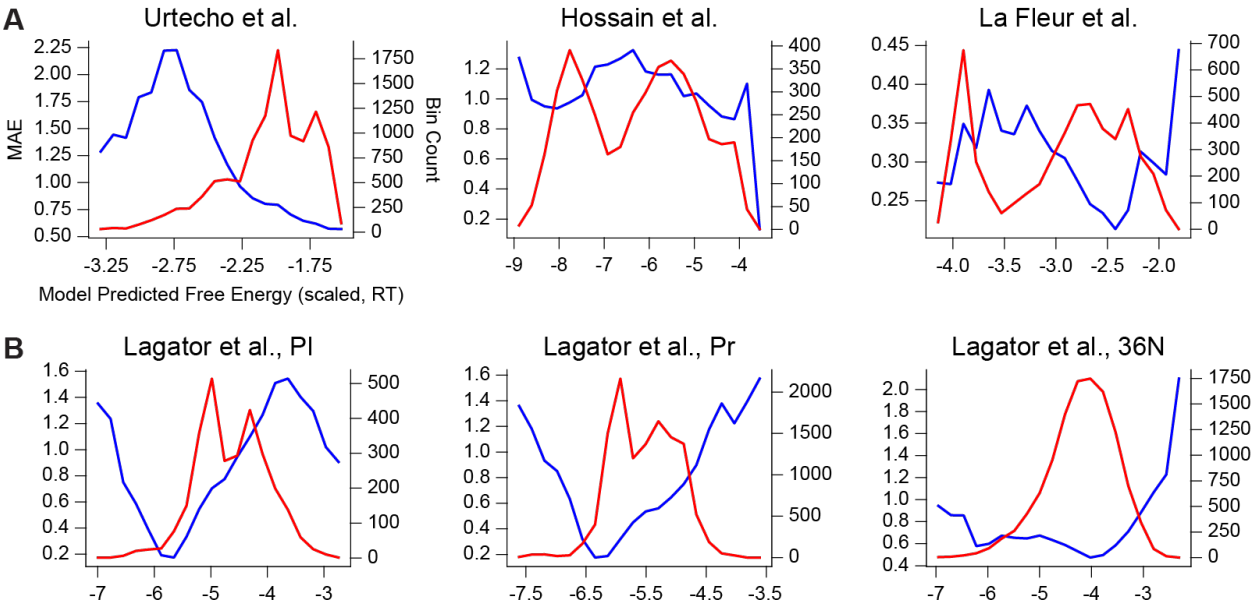
Supplementary Figure 1: Replicate Correlations. RNA read count correlations for (**A**) replicate 1 vs replicate 2, (**B**) replicate 1 vs replicate 3 and (**C**) replicate 2 vs replicate 3 (R^2 = 0.94, 0.97, and 0.99 respectively). DNA read count correlations for (**D**) replicate 1 vs replicate 2, (**E**) replicate 1 vs replicate 3 and (**F**) replicate 2 vs replicate 3 (R^2 = 0.99, 0.99, and 0.99 respectively). Transcription Initiation Rate correlations for (**G**) replicate 1 vs replicate 2, (**H**) replicate 1 vs replicate 3 and (**I**) replicate 2 vs replicate 3 (R^2 = 0.89, 0.94, and 0.97 respectively). Blue dots represent -10 extended promoter variants, orange dots represent -35 hexamer promoter variants, green dots represent -10 hexamer promoter variants, pink dots represent UP element promoter variants, brown dots represent discriminator promoter variants, purple dots represent spacer promoter variants, and grey dots represent ITR promoter variants. Data are provided in **Supplementary Data 1**.



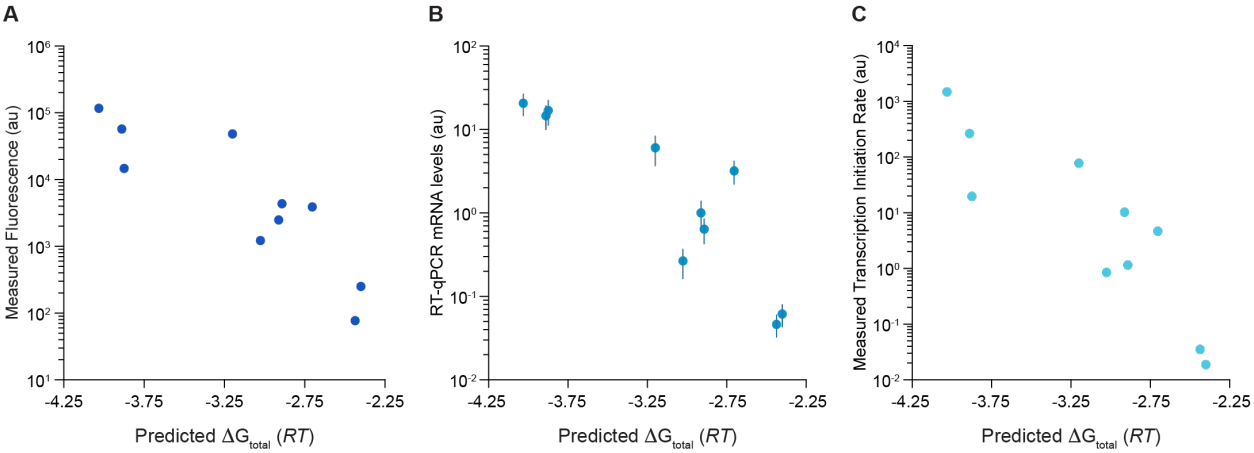
Supplementary Figure 2: Transcription Start Site Profiles. (**A**) The measured TSS distribution of a variant that met all filtering criteria and exhibited a strong on-target peak. (**B**) The measured TSS distribution of a variant that did not meet the minimum read threshold. (**C**) The measured TSS distribution of a variant that had an off-target peak (position ~105) with greater than twice the counts of the on-target peak (position 53). (**D**) The measured TSS distribution of a variant with multiple strong TSS peaks. Data are provided in **Supplementary Data 1**.



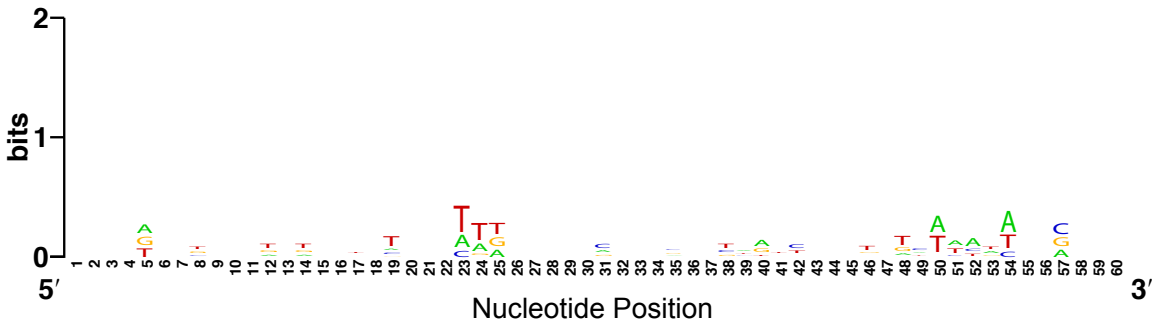
Supplementary Figure 3: Model Predictions on IPTG-inducible Promoters. LaFleur linear model free energy predictions are compare to measured transcription rates for 1493 genome-integrated IPTG-inducible promoters, characterized *in vivo* by Yu et al. with 1 mM IPTG added [17] (R^2 = 0.65, Spearman's ρ = 0.70). Data are provided in **Supplementary Data 1**.



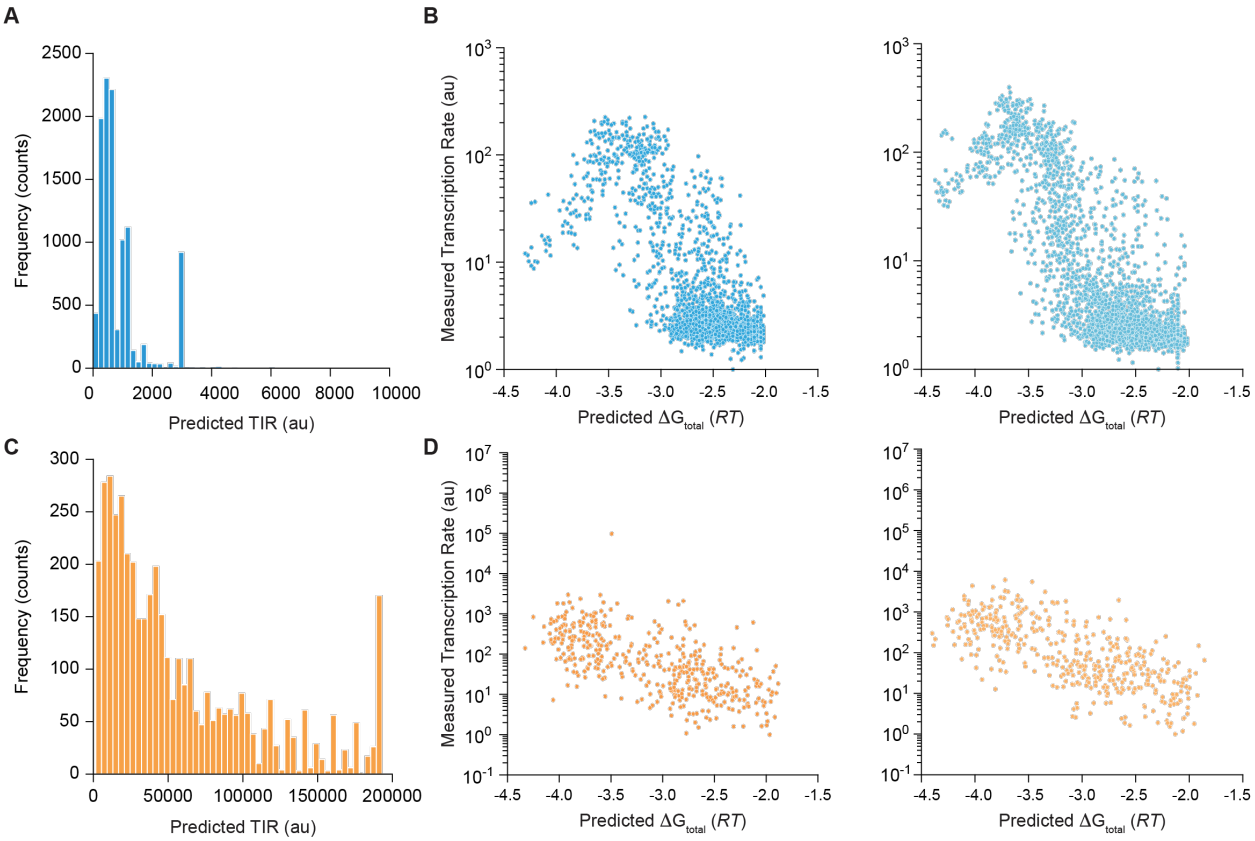
Supplementary Figure 4: Model Residuals Across Transcription Space. (**A**) LaFleur linear model residual distributions across 20 evenly spaced free energy bins for Urtecho et al. (left), Hossain et al. (middle) and LaFleur et al. (right). (**B**) LaFleur linear model residual distributions across 20 evenly spaced free energy bins for the PI dataset from Lagator et al. (left), Pr dataset from Lagator et al. (middle) and 36N dataset from Lagator et al. (right). Blue lines are MAE values which correspond to the left y-axis, red lines are the number of variants in each bin which correspond to the right y-axis. Data are provided in **Supplementary Data 4**.



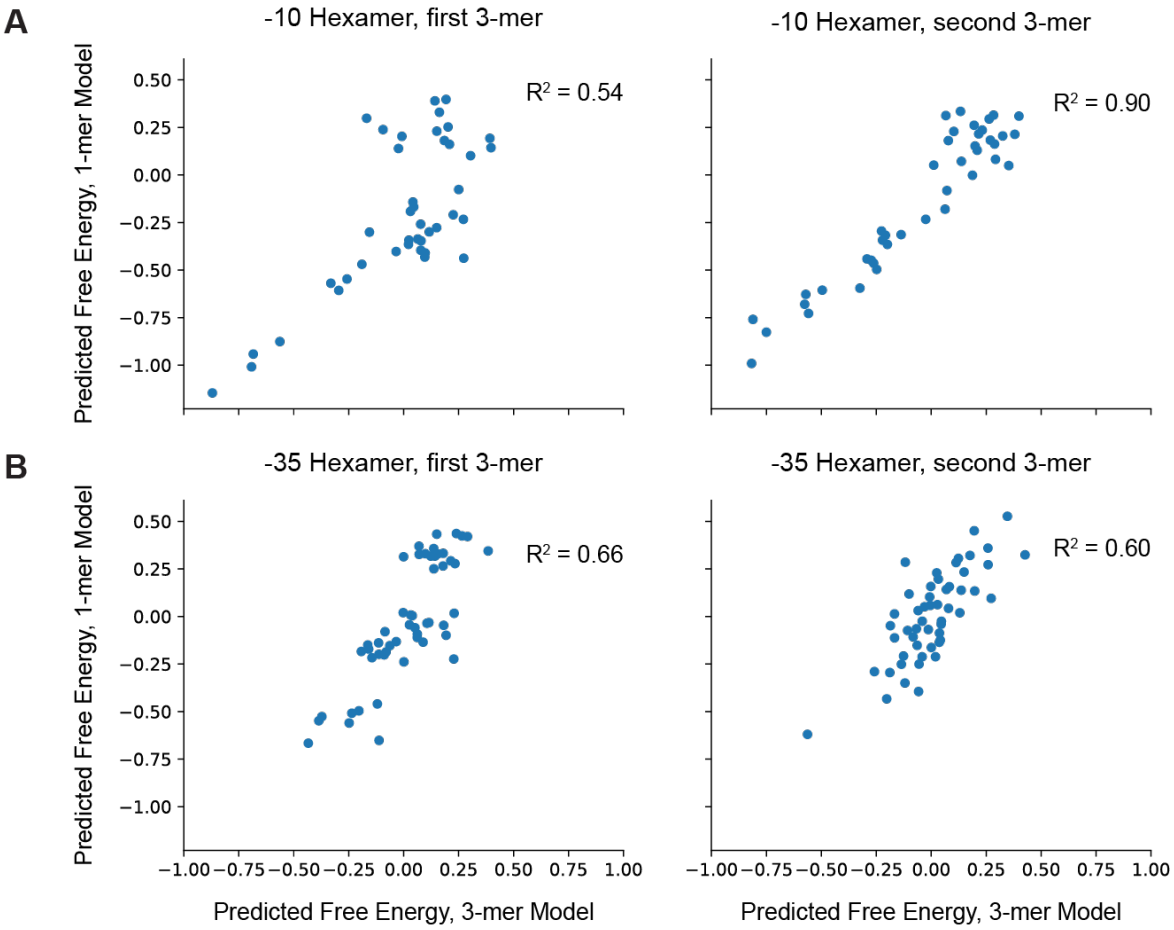
Supplementary Figure 5: Model Accuracy on Selected Non-Repetitive Promoters with 3 Types of Measurements. (**A**) Model predictions are compared to transcription rate measurements for 10 promoters characterized by Hossain et al. using flow cytometry (R^2 = 0.75, Spearman's ρ = 0.87). (**B**) Model predictions are compared to transcription rate measurements for 10 promoters characterized by Hossain et al. using RT-qPCR (R^2 = 0.78, Spearman's ρ = 0.87). (**C**) Model predictions are compared to transcription rate measurements for 10 promoters characterized by Hossain et al. using read-based measurements. (R^2 = 0.77, Spearman's ρ = 0.90). Dots and error bars represent the mean ± standard deviation for triplicate measurements (n = 3 biological replicates). Data are provided in **Supplementary Data 1**.



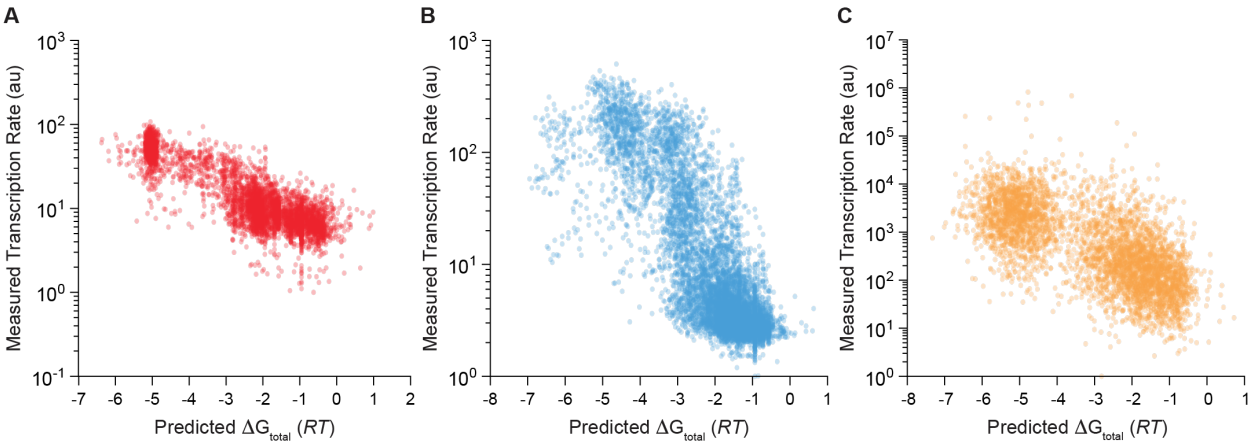
Supplementary Figure 6: Sequence Entropy for 60bp Forward Engineered Promoters. Sequence entropy analysis for 60 bp promoters showed that forward engineered promoters exhibit low levels of similarity. Promoters, on average, shared a ~27% sequence similarity and had an average pair-wise hamming distance of 44 bp. Data are provided in **Supplementary Data 1**.



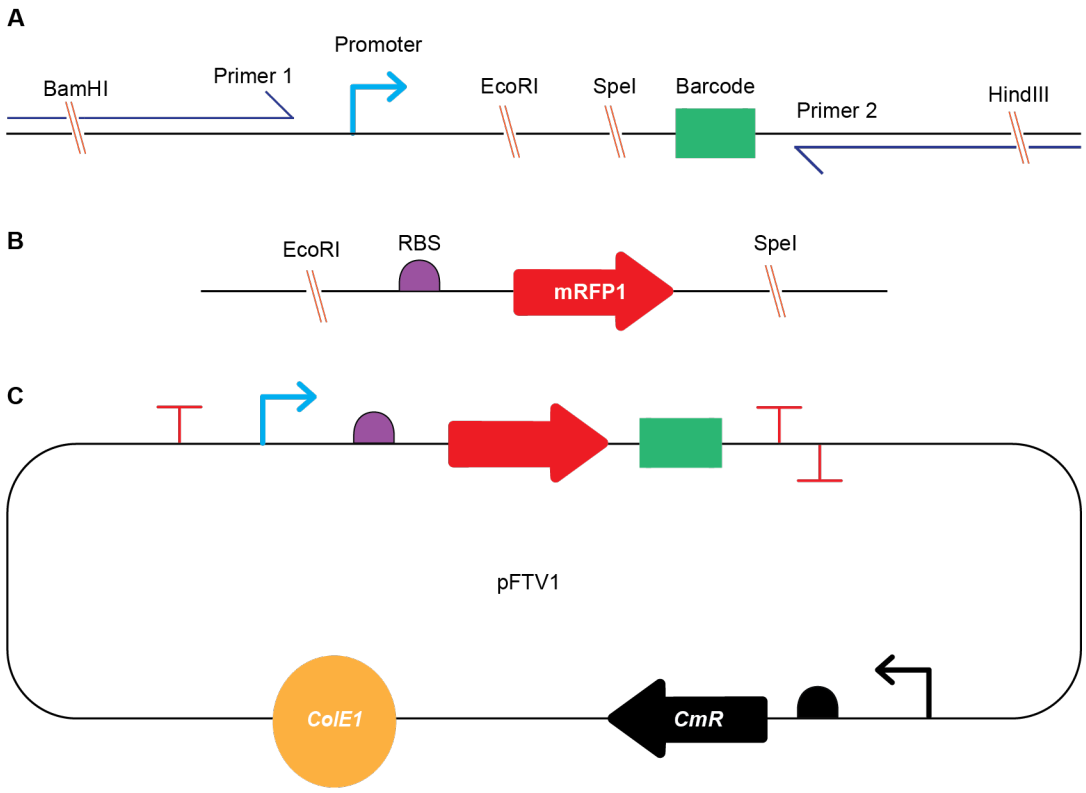
Supplementary Figure 7: mRNA Decay Alters RNA Level Measurements. (**A**) Predicted translation initiation rate (TIR) distribution for 10898 genome-integrated promoters, exhibiting a 1679-fold dynamic range. (**B**) Model predictions on 2716 low TIR promoters (left) and 2719 high TIR promoters (right). R^2 is equal to 0.51 (left) and 0.61 (right). (**C**) Predicted translation initiation rate (TIR) distribution for 4350 plasmid-endoded promoters, exhibiting a 2680-fold dynamic range. (**D**) Model predictions on 435 low TIR promoters (left) and 430 high TIR promoters (right). R^2 is equal to 0.43 (left) and 0.50 (right). Data are provided in **Supplementary Data 1**.



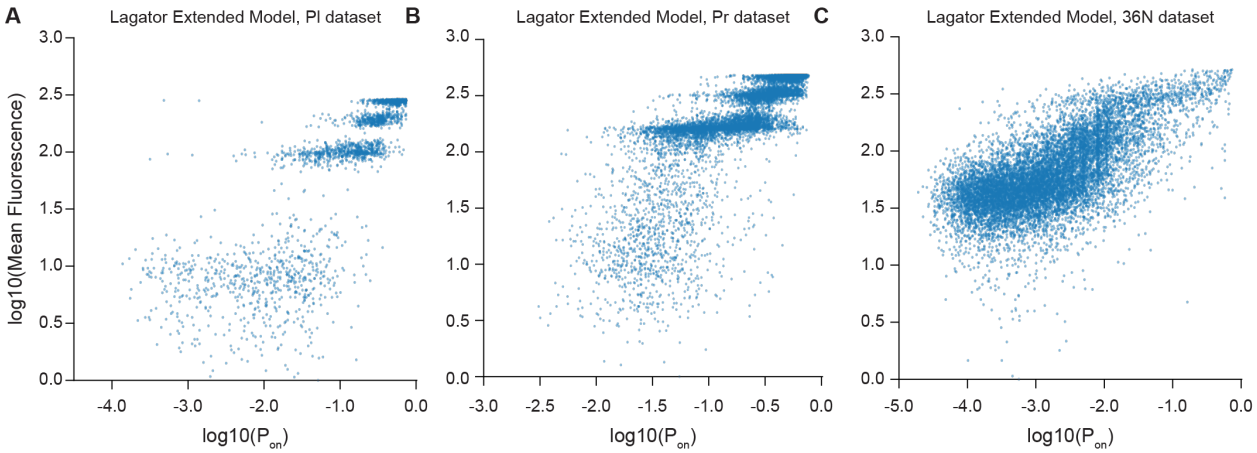
Supplementary Figure 8: Hexamer Model Comparison (1-mer vs. 3-mer). Correlation between predicted free energies of the single-nt model and tri-nt model for the (**A**) -10 hexamer and (**B**) -35 hexamer. For the single-nt model, 1-mer contributions are summed to calculate the free energy of each 3-mer, then plotted against model coefficients from the tri-nt model. Hexamers are broken up into 2 adjacent non-overlapping 3-mers. Squared Pearson correlation coefficients are reported. Data are provided in **Supplementary Data 4**.



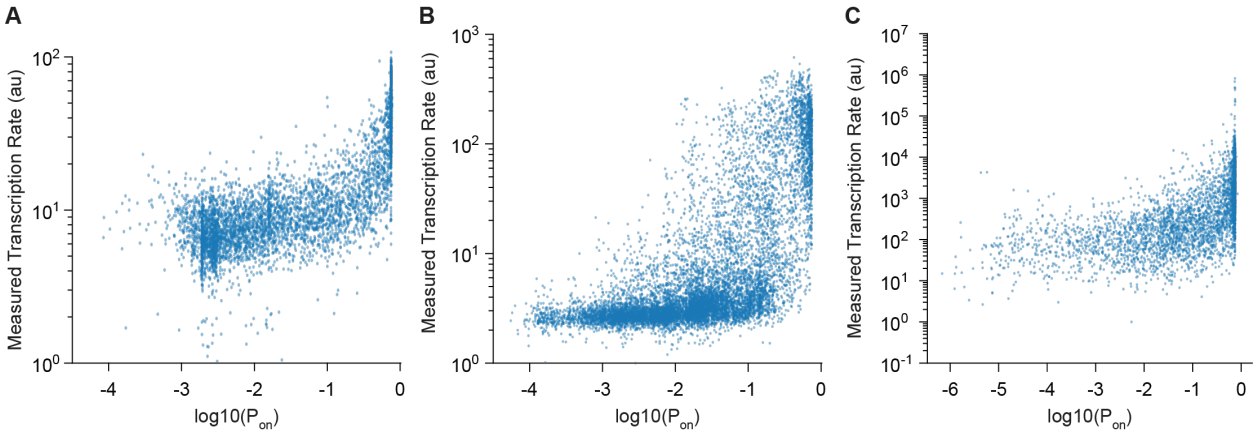
Supplementary Figure 9: A Quadratic Non-Linear Free Energy Model. (**A**) A non-linear free energy model incorporating all possible pair-wise interactions between promoter motifs was developed with 30 quadratic terms. Non-linear model predictions for 5391 designed promoters (this study) are compared to *in vitro* transcription rate measurements (R^2 = 0.76). (**B**) Non-linear model predictions for 10898 genome-integrated promoters are compared to *in vivo* transcription rate measurements (R^2 = 0.69). (**C**) Non-linear model predictions on 4350 non-repetitive plasmid-encoded promoters are compared to *in vivo* transcription rate measurements (R^2 = 0.46). Data are provided in **Supplementary Data 2**.



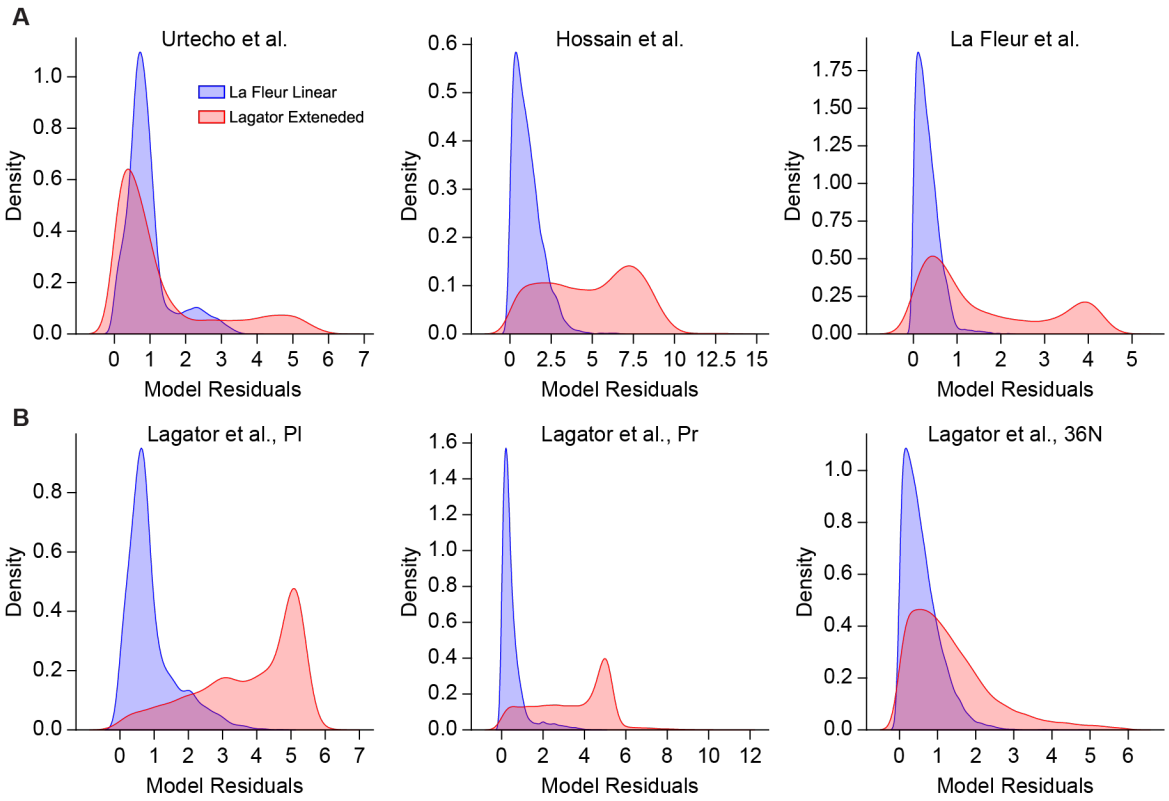
Supplementary Figure 10: Oligo Designs and Plasmid Map. (**A**) Oligopool library design with variable promoters. Each library variant contains two primer bindings sites with internal restriction enzyme cut sites (navy), a unique promoter, two additional restriction enzyme cut sites, and a unique barcode. (**B**) A Gene-block containing two restriction cut sites, a ribosome binding site (5k, RBS Calculator 2.1) and a reporter protein. (**C**) The final assembled plasmid pool used for promoter library characterization.



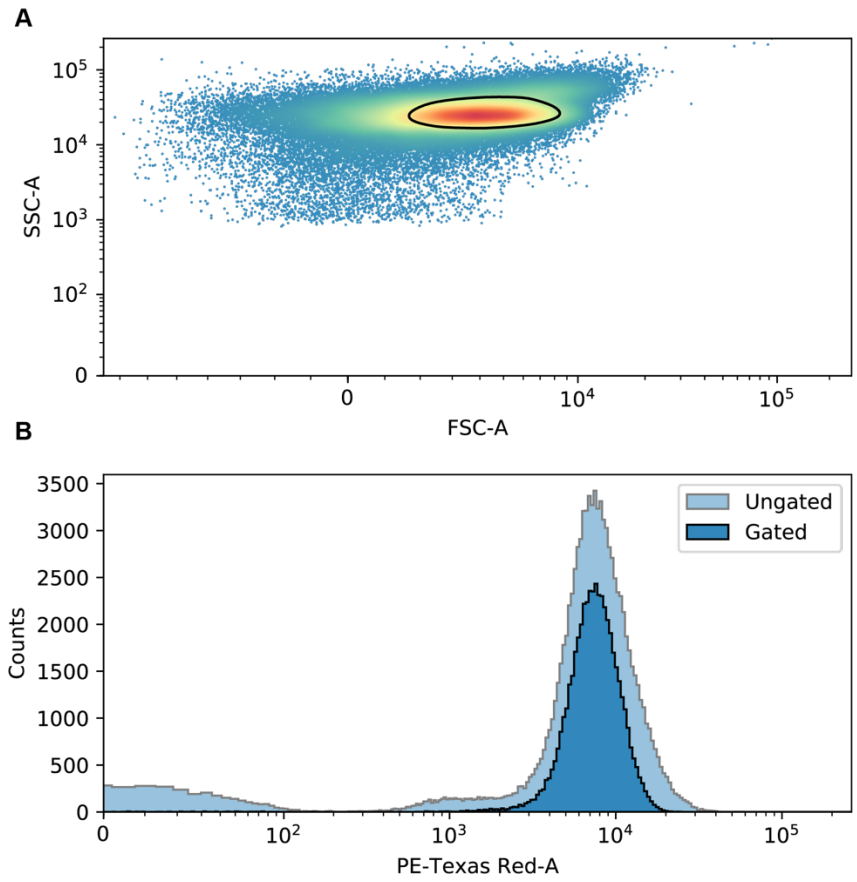
Supplementary Figure 11: Lagator Extended Model Predictions on Lagator Datasets. (**A**) Lagator extended model predictions for 2903 PI mutant promoters are compared to reported *in vivo* Sort-Seq means (R^2 = 0.73). (**B**) Lagator extended model predictions for 12194 Pr mutant promoters are compared to reported *in vivo* Sort-Seq means (R^2 = 0.67). (**C**) Lagator extended model predictions for 11523 random 36N sequences are compared to reported *in vivo* Sort-Seq means (R^2 = 0.44). Data are provided in **Supplementary Data 3**.



Supplementary Figure 12: Lagator Extended Model Validation. (**A**) Lagator extended model predictions for 5391 designed promoters (LaFleur et al., this study) are compared to *in vitro* transcription rate measurements (R^2 = 0.60). (**B**) Lagator extended model predictions for 10898 genome-integrated promoters (Urtecho et al.) are compared to *in vivo* transcription rate measurements (R^2 = 0.45). (**C**) Lagator extended model predictions on 4350 non-repetitive plasmid-encoded promoters (Hossain et al.) are compared to *in vivo* transcription rate measurements (R^2 = 0.39). Data are provided in **Supplementary Data 3**.



Supplementary Figure 13: Slope-only Model Benchmarking, Residual Distributions. (**A**) Model residual distributions for Urtecho et al. (left), Hossain et al. (middle) and LaFleur et al. (right). (**B**) Model residual distributions for the Pl dataset from Lagator et al. (left), Pr dataset from Lagator et al. (middle) and 36N dataset from Lagator et al. (right). Blue distributions are from the LaFleur et al. linear model, red distributions are from the Lagator et al. extended model. Model residuals are the absolute difference between the model prediction [ΔG_{total} for LaFleur and $\log(P_{on})$ for Lagator] and the natural log of the measured transcription rates, using a best-fit slope relationship for each model and dataset. Data are provided in **Supplementary Data 1 & 3**. Best fit parameters are provided in **Supplementary Data 5**.



Supplementary Figure 14: Flow Cytometry Gating. (**A**) 2D density plot showing the gating strategy used in this study. Forward scattering and side scattering were used to identify events corresponding to *E. coli* cells, and the densest area (yellow and red) was used in calculating the mean fluorescence. (**B**) Texas Red intensity for the gated and ungated events.

Supplementary Table 1: Model Features.

A report of the total number of model features used for each promoter region, and the total number of features retained after feature reduction.

Feature Name, Feature Type	Number of Parameters	Number Retained[a]
-10 hexamer 3-mers	128	128
-35 hexamer 3-mers	128	128
Discriminator 3-mers	128	64
-10 extended 2-mers	32	16
Discriminator Length	7	0
Discriminator GC Content	1	0
Discriminator Purine Content	1	0
TSS 2-mers	16	0
Spacer Length	6	6
Spacer Stacking Free Energy	1	0
Spacer Torsional Energy	1	0
UP Groove Width (Distal and Proximal)	2	2
ITR Purine Content	1	0
ITR Pause-Element Location	15	0
UP A-tract Length (Distal and Proximal)	2	0
UP AT Content	1	0
DNA Rigidity	1	1
R-loop Strength	1	1

[a] *Retained parameters are the only ones present in the final model*

Supplementary Table 2: Model Selection.

A summary of the selection criteria used for converging on the final model version. Included are models with the original set of features (472) as well as models with the reduced set of features (346). Criteria for model selection was a high Pearson coefficient on the test set and 8 correct positive controls.

Model Type	Number of Features	R^2 Train	MAE Train	MSE Train	R^2 Test	MAE Test	MSE Test	Correct / Total Positive Controls[a]
Ridge	472	0.82	0.265	0.120	0.80	0.270	0.126	4/8
Lasso	472	0.82	0.264	0.120	0.80	0.270	0.126	4/8
Elastic Net	472	0.82	0.264	0.120	0.80	0.270	0.126	4/8
Ridge	346	0.80	0.274	0.133	0.80	0.281	0.131	8/8
Lasso	346	0.80	0.274	0.134	0.80	0.281	0.131	8/8
Elastic Net	346	0.80	0.274	0.133	0.80	0.281	0.131	8/8

[a] *Positive controls were defined to match known consensus promoter sequence motifs* [38]

Supplementary Table 3: Primers, RNA Adapter, and DNA Fragment Sequences.

A list of the nucleic acid sequences used during cloning.

Part Name	Part Sequence
primer 1	TGCTGGATCCCTACTCTGAG
primer 2	CTATAAGCTTGGTCCGACGG
primer 3	TCCCACAACGAAGACTACACC
primer 4	CTCTTTGATAACGTCTTCAGAGC
primer 5	AACAGGATCCACGCACTCTA
primer 6	CGGACGACCTTCACCTTCA
gene-block 1	CTGTGCGTTAGGTGATGCCGCGGCTGTACCGCCCATGTGCTCACGAATTCGAT CAAATTTCGAGGTTCCATATGGCGAGCTCTGAAGACGTTATCAAAGAGTTCATG CGTTTCAAAGTTCGTATGGAAGGTTCCGTTAACGGTCACGAGTTCGAAATCGAA GGTGAAGGTGAAGGTCGTCCGTACGAAGGTACCCAGACCGCTAAACTGAAAGT TACCAAAGGTGGTCCGCTGCCGTTCGCTTGGGACATCCTGTCCCCGCAGTTCC AGTACGGTTCCAAAGCTTACGTTAAACACCCGGCTGACATCCCGGACTACCTG AAACTGTCCTTCCCGGAAGGTTTCAAATGGGAACGTGTTATGAACTTCGAAGAC GGTGGTGTTGTTACCGTTACCCAGGACTCCTCCCTGCAAGACGGTGAGTTCAT CTACAAAGTTAAACTGCGTGGTACCAACTTCCCGTCCGACGGTCCGGTTATGCA GAAAAAAACCATGGGTTGGGAAGCTTCCACCGAACGTATGTACCCGGAAGACG GTGCTCTGAAAGGTGAAATCAAAATGCGTCTGAAACTGAAAGACGGTGGTCACT ACGACGCTGAAGTTAAAACCACCTACATGGCTAAAAAACCGGTTCAGCTGCCG GGTGCTTACAAAACCGACATCAAACTGGACATCACCTCCCACAACGAAGACTAC ACCATCGTTGAACAGTACGAACGTGCTGAAGGTCGTCACTCCACCGGTGCTTA ATAAACTAGTAAACGCAGTTACCCCATAGGCT
RNA adapter	AACAGGAUCCACGCACUCUANN

Supplementary Table 4: Model Benchmarking – Slope Only.

Model benchmarking with a best fit slope. Columns represent the LaFleur linear model, the LaFleur quadratic model, the Lagator standard model, the Lagator extended model, and the number of samples in each dataset. Rows correspond to performance metrics for each dataset.

	LaFleur (linear)	LaFleur (quadratic)	Lagator (standard)	Lagator (extended)	N samples
R^2 Urtecho[a]	0.60	0.69	0.47	0.45	10,898
MAE Urtecho[a]	0.93	0.56	1.32	1.33	
MSE Urtecho[a]	1.28	0.59	3.93	3.99	
R^2 Hossain[a]	0.45	0.46	0.42	0.39	4,350
MAE Hossain[a]	1.08	1.96	4.83	4.85	
MSE Hossain[a]	1.88	5.66	31.5	31.0	
R^2 LaFleur	0.79	0.76	0.58	0.60	5,391
MAE LaFleur	0.33	0.79	1.43	1.56	
MSE LaFleur	0.18	0.88	3.98	4.27	
R^2 Lagator Pl	0.47	0.49	0.67	0.73	2,903
MAE Lagator Pl	0.91	0.98	3.53	3.74	
MSE Lagator Pl	1.34	1.70	14.5	16.1	
R^2 Lagator Pr	0.35	0.37	0.55	0.67	12,194
MAE Lagator Pr	0.51	1.06	3.14	3.40	
MSE Lagator Pr	0.62	1.79	13.1	14.7	
R^2 Lagator 36N	0.19	0.15	0.33	0.44	11,523
MAE Lagator 36N	0.59	1.71	1.16	1.34	
MSE Lagator 36N	0.57	4.50	2.31	3.00	

[a] *Rows with green shading indicate unseen datasets to both the LaFleur et al. and Lagator et al. models during training. See Discussion for details. Best fit parameters are provided in* ***Supplementary Data 5****.*

Supplementary Table 5: Model Benchmarking –Intercept Only.

Model benchmarking with a best fit intercept. Columns represent the LaFleur linear model, the LaFleur quadratic model, the Lagator standard model, the Lagator extended model, and the number of samples in each dataset. Rows correspond to performance metrics for each dataset.

	LaFleur (linear)	LaFleur (quadratic)	Lagator (standard)	Lagator (extended)	N samples
R^2 Urtecho[a]	0.60	0.69	0.47	0.45	10,898
MAE Urtecho[a]	0.82	0.56	1.95	1.24	
MSE Urtecho[a]	1.12	0.59	5.47	2.39	
R^2 Hossain[a]	0.45	0.46	0.42	0.39	4,350
MAE Hossain[a]	1.20	1.12	2.17	1.60	
MSE Hossain[a]	2.25	2.08	8.65	4.79	
R^2 LaFleur	0.79	0.76	0.58	0.60	5,391
MAE LaFleur	0.33	0.73	2.70	1.60	
MSE LaFleur	0.17	0.80	8.86	3.18	
R^2 Lagator PI	0.47	0.49	0.67	0.73	2,903
MAE Lagator PI	1.01	0.79	1.29	0.66	
MSE Lagator PI	1.58	1.13	3.25	0.98	
R^2 Lagator Pr	0.35	0.37	0.55	0.67	12,194
MAE Lagator Pr	0.54	0.55	0.94	0.41	
MSE Lagator Pr	0.71	0.64	1.52	0.37	
R^2 Lagator 36N	0.19	0.15	0.33	0.44	11,523
MAE Lagator 36N	0.52	0.57	1.59	1.22	
MSE Lagator 36N	0.46	0.55	4.02	2.29	

[a] *Rows with green shading indicate unseen datasets to both the LaFleur et al. and Lagator et al. models during training. See Discussion for details. Best fit parameters are provided in* ***Supplementary Data 5****.*

Supplementary Table 6: Model Benchmarking – Slope and Intercept.

Model benchmarking with a best fit slope and intercept. Columns represent the LaFleur linear model, the LaFleur quadratic model, the Lagator standard model, the Lagator extended model, and the number of samples in each dataset. Rows correspond to performance metrics for each dataset.

	LaFleur (linear)	LaFleur (quadratic)	Lagator (standard)	Lagator (extended)	N samples
R^2 Urtecho[a]	0.60	0.69	0.47	0.45	10,898
MAE Urtecho[a]	0.68	0.56	0.78	0.81	
MSE Urtecho[a]	0.76	0.59	0.99	1.04	
R^2 Hossain[a]	0.45	0.46	0.42	0.39	4,350
MAE Hossain[a]	1.08	1.07	1.13	1.16	
MSE Hossain[a]	1.88	1.86	2.00	2.12	
R^2 LaFleur	0.79	0.76	0.58	0.60	5,391
MAE LaFleur	0.29	0.32	0.42	0.42	
MSE LaFleur	0.15	0.17	0.30	0.28	
R^2 Lagator PI	0.47	0.49	0.67	0.73	2,903
MAE Lagator PI	0.85	0.78	0.57	0.49	
MSE Lagator PI	1.18	1.13	0.73	0.59	
R^2 Lagator Pr	0.35	0.37	0.55	0.67	12,194
MAE Lagator Pr	0.51	0.50	0.39	0.33	
MSE Lagator Pr	0.62	0.61	0.42	0.31	
R^2 Lagator 36N	0.19	0.15	0.33	0.44	11,523
MAE Lagator 36N	0.52	0.54	0.47	0.43	
MSE Lagator 36N	0.46	0.48	0.38	0.31	

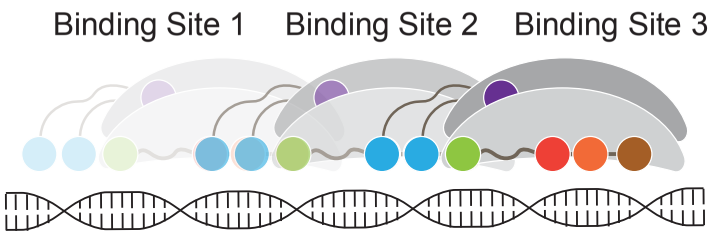
[a] *Rows with green shading indicate unseen datasets to both the LaFleur et al. and Lagator et al. models during training. See Discussion for details. Best fit parameters are provided in* ***Supplementary Data 5****.*

The Promoter Calculator

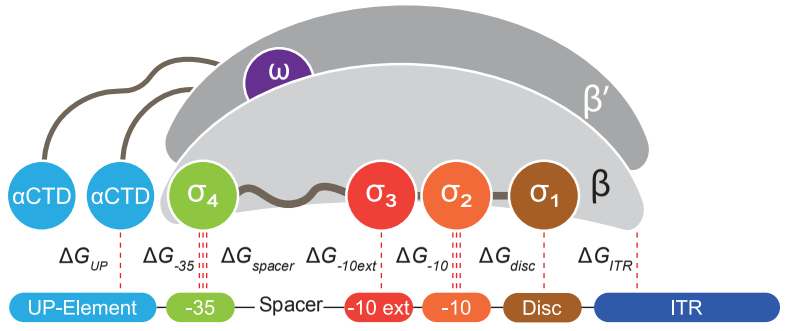
a statistical thermodynamic model of transcription initiation in bacteria

Genetic System Sequence ⟶ Transcriptional Profile

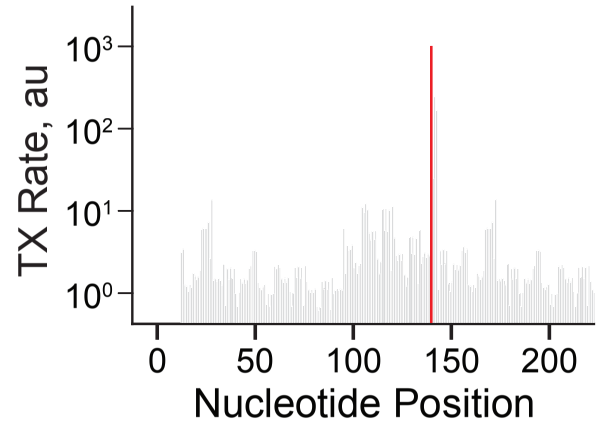
List RNAP/σ Binding Sites



Calculate Total Free Energies



Predict Transcription Rates



Free Energy Model for *Escherichia coli* RNAP/σ^{70} Interactions with DNA

-35 Hexamer

ΔG_{-35}

first 3 nt	second 3 nt
TTG	TGC
TTT	AAT
ATG	CCG
TAG	CGG
TGG	TAC
TTC	TCC
CTG	CAG
GTG	GAC
GTA	TCA
TCT	GGT
CTA	CTC
TAT	ACG
TTA	AGG
TCG	GCT
TGA	CAT
CTT	CCT
TAA	CAC
GTC	AAC
GTT	CGA
TGT	GAG
ATC	CCA
GCG	GGC
ATT	AAG
CGT	CAA
AAA	ATA
AGC	GGG
GGA	GCC
GAT	GAA
AGA	ACA
AGT	CCC
ACT	CGC
ACC	GCA

-10 Hexamer

ΔG_{-10}

first 3 nt	second 3 nt
ACA	TGG
ACT	TTG
CCT	AAG
AAT	CTG
CCA	CGG
CAA	GAT
ATC	GAG
AAA	ATG
GTA	AAC
TAA	CGA
ACG	CCC
ATT	TTA
AGA	TGA
CTA	AGG
TGT	GTT
TTT	GTG
GAA	TCG
TCT	AGT
TCA	GGA
CTT	TGC
CAT	CTC
TAT	CGC
ATA	TAC
CAG	AGC
GCA	TTC
GCT	GCC
CCG	GGG
ACC	GTC
TAG	GAC
CAC	TCC
CGT	GGC
GCG	GGT

Discriminator

ΔG_{disc}

first 3 nt	second 3 nt
TAT	ACA
TAA	CCG
TAC	GCG
TAG	GGT
AAT	TGC
CAT	TCC
GAT	AAC
GTA	TGT
CAA	TTG
TTA	TGG
CTA	CGA
CAC	TCT
ATT	TGA
GAC	CAG
GGG	AAA
CTG	GAA
GGC	AAG
CGG	GAG
CGC	ACT
GTC	CCA
GGA	GCA
GTG	TCA
CTC	AGT
GCC	CCC
ATG	GCT
ATC	GTT
ATA	TTC
AGG	TCG
AGC	TTT
AGA	CTT
ACG	CGT
ACC	CCT

-10 Extended

ΔG_{-10ext}

2 nt motif
AAT
AAG
TAT
CTA
ACT
TTA
AGT
AGA
CAT
TGA
ATT
GTA
GAT
CTC
TCT
AGC
GCT
GAA
TGT
ACG
CCT
TAC
ATA
CAC
GGT
GAC
TTT
GCC
GTT
CCG
CGT
GCG
CTT
GCA
ACA
TCG
AAA
GTC
AGG
CCC
TTC
CAG
AAC
TCG
ATG
TTG
CTG
GGA
CGG
TCA
GGG
GAG
CGA
GTG
TGG
GGC
TAG
CAA
ATC
CCA
ACC
TGC
TAA
CGC

GCT	AAT
GCA	TAG
CGC	GGC
TCT	AGG
CCT	GTG
TCC	ACA
TAT	CTC
CAC	AAC
GCG	TTC
CGA	TAC
TCG	CGG
ACT	GAT
CTG	GTC
ACC	TAA
CCG	GGG
CCC	TTA
TCA	AAG
GCC	CAA
AGT	TGA
CCA	CGT
CTA	AGA
CAT	CAG
TGC	TTT
ATA	GAA
TTG	ATC
AAA	GTT
GGA	AGC
TGT	GTA
ATT	TGG
ACG	CTT
GAC	ATG
GGT	GAG

TG	AT	CA	TA	TT	GT	AG	CC
GG	AA	CG	GA	AC	GC	CT	TC

UP

ΔG_{UP}

Groove Width Distal				
50	119	126	198	225

Groove Width Proximal				
50	119	126	198	225

AT Content		
High	Med	Low

ITR

ΔG_{ITR}

RNA:DNA loop free energy

-1.50 0 3.0

Spacer

ΔG_{spacer}

Length

15	16	17	18	19	20

-1.0 -0.5 0.0 0.5 1.0

Strong — Weak

Interaction Energy

$$\Delta G_{total} = \Delta G_{UP} + \Delta G_{-35} + \Delta G_{spacer} + \Delta G_{-10ext} + \Delta G_{-10} + \Delta G_{disc} + \Delta G_{ITR}$$

$$TX = TX_{ref} \times e^{-\beta(\Delta G_{total} - \Delta G_{total,ref})}$$